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National Toxicology Program

Multigenerational Reproductive Toxicology (TR 547) and Toxicology and Carcinogenesis (TR 548) Studies of Ethinyl Estradiol in Sprague-Dawley Rats

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Outline

- Background on NTP's endocrine disruptor studies and ethinyl estradiol
- Summary of dose range finding study (data presented in TR 547)
- Multigeneration study results (TR 547)
- Chronic study results (TR 548)

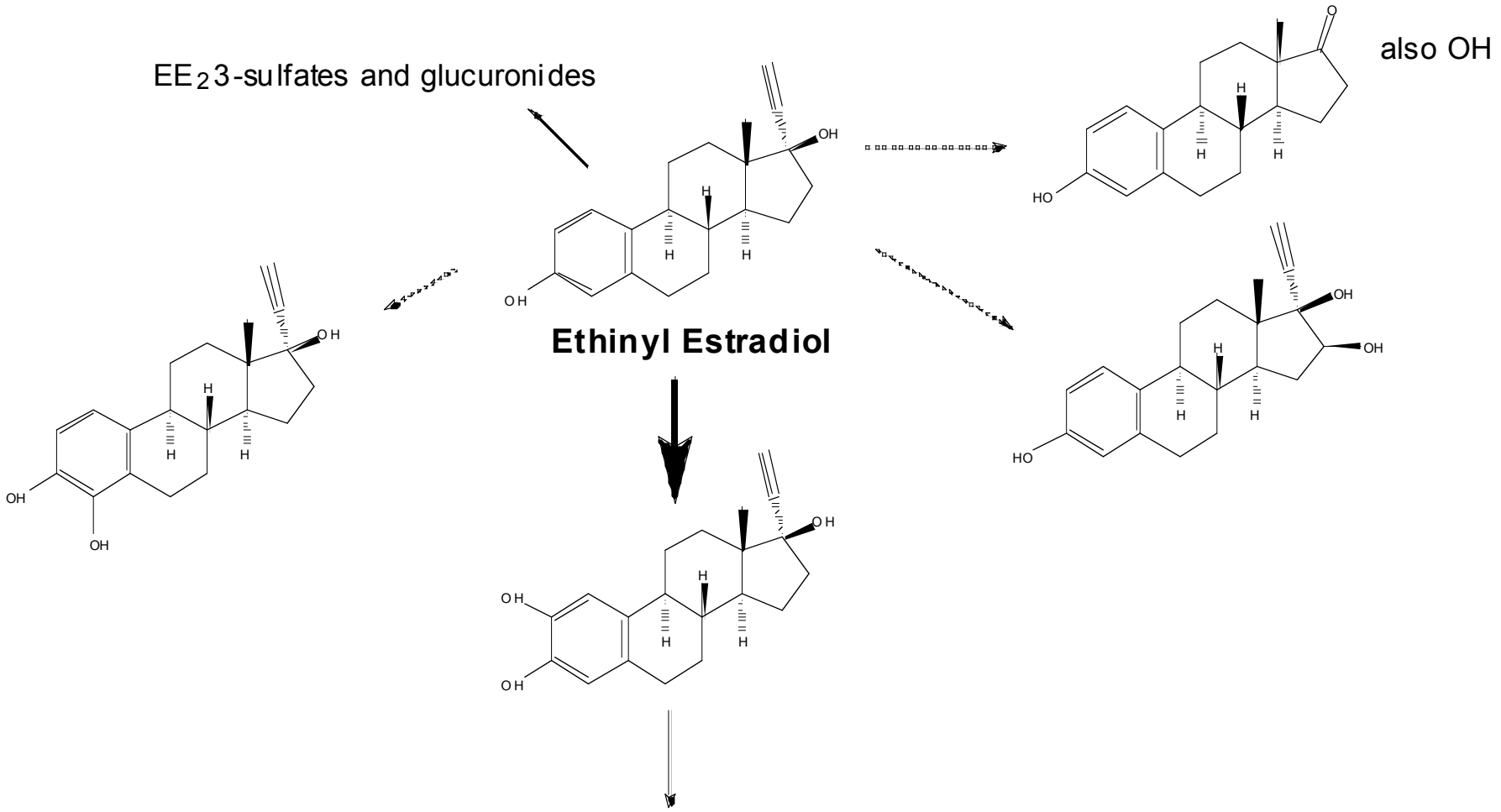
Background

- Endocrine Disruptor Evaluations Conducted Under NIEHS/NTP-FDA/NCTR Interagency Agreement
  - Evaluate long term effects of a series of compounds of varying potencies (genistein, nonylphenol, ethinyl estradiol, vinclozolin, methoxychlor)
  - Multigeneration reproductive studies with differing exposure windows across generations to evaluate the possibility of magnification of subtle effects across generations, reversibility
  - Include doses within likely human exposure range and/or below reported NOAEL
  - Chronic effects following exposure during different exposure windows
  - Original plan called for testing of pure compounds with consideration of subsequent testing more complex mixtures (e.g. soy extract, chlorinated hydrocarbons identified in human breast milk)

Background – Ethinyl Estradiol (EE_2)

- Potent synthetic estrogen primarily used as the estrogenic component of oral contraceptives due to oral bioavailability
- Found as contaminant in aquatic environment
- Somewhat selective for ERα
- Selected as potent estrogen for comparison with the weaker estrogens (genistein, *p*-nonylphenol) tested

Ethinyl Estradiol Metabolism (Partial)



Dose Range Finding Study Design

- Test Animal: CD (Sprague-Dawley) rat (NCTR colony)
- Exposure Window: GD 7 through PND 50/63/77
- Route of Exposure: Diet, Purina 5K96
- Control and 6 doses (0, 0.1, 1, 5, 25, 100, and 200 ppb) for reproductive study, control and 3 doses for other endpoints
- Five litters per dose group, standardized litters (4 pups/sex/litter)
- Goal: To select doses causing reproductive tract effects in pups that would not be likely to severely impair reproduction in the F_1 generation of the multigeneration study

Summary of EE_2 effects – Reproductive DRF Study (0, 0.1, 1, 5, 25, 100, 200 ppb)

Lowest Effective Dose (ppb)	Observation
5	**Males:** preputial separation acceleration, dorsolateral prostate weight increase
25	**Females:** vaginal opening acceleration (trend) **Males:** mammary hyperplasia
100	**Females:** decreased dam body weight and feed consumption, birth weight decrease, anestrus (ovary) **Males:** birth weight decrease, spermatocyte/spermatatid degeneration, depletion of secretory material (seminal vesicles), mineralization (renal tubules)
200	**Females:** Pup body weight and food consumption/ decrease; vaginal opening acceleration; ovary weight decrease, liver weight (adjusted for body weight) increase; vaginal atrophy, mucocyte metaplasia, and dystrophy **Males:** Preputial separation, delay; testis and ventral prostate weight/ decrease; pituitary weight (adjusted for body weight)/ increase; pup body weight and food consumption/ decrease; testicular spermatid head count decrease

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Multigenerational Reproductive Toxicology Study of Ethinyl Estradiol in Sprague-Dawley Rats

TR 547

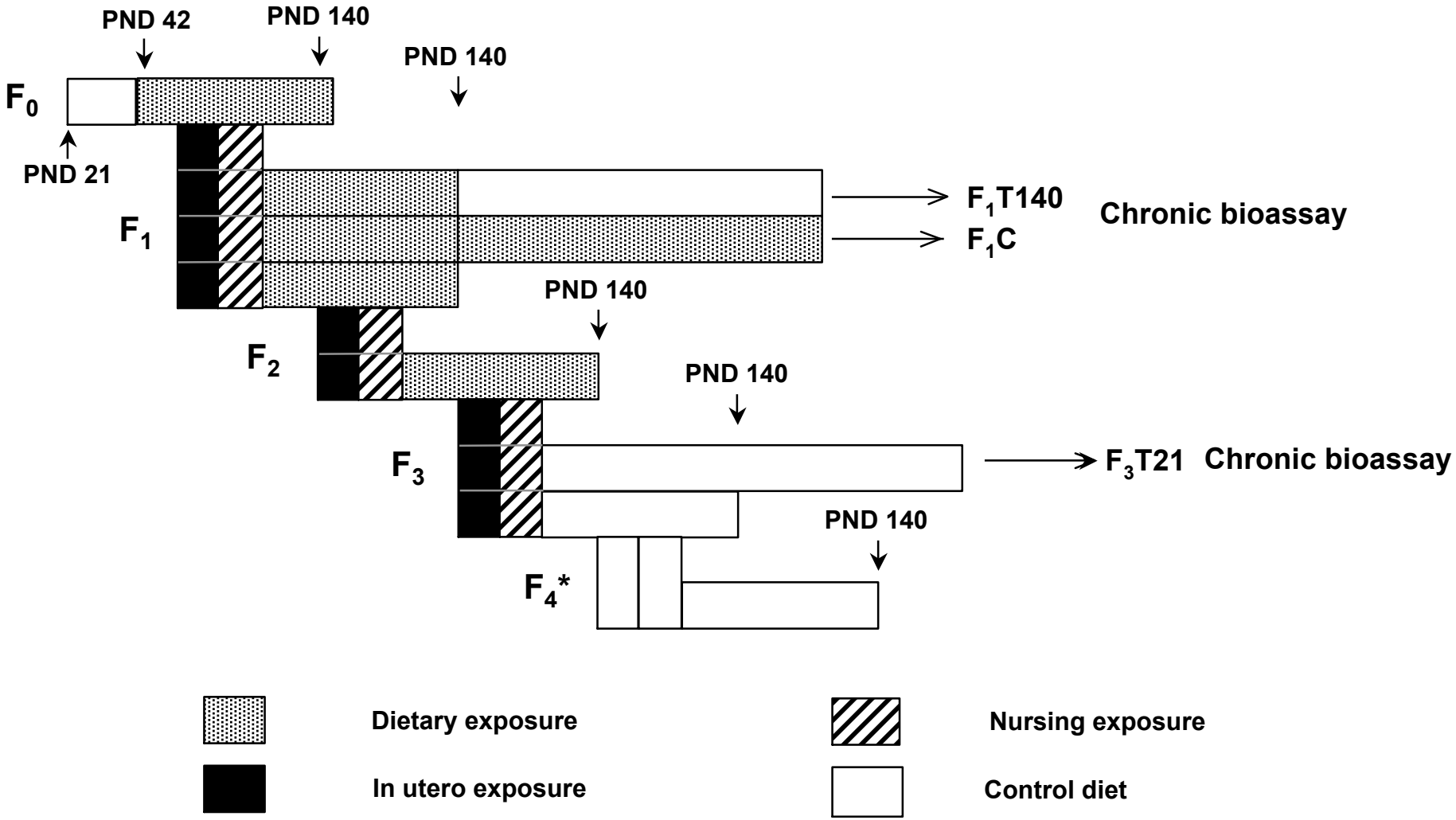
Multigeneration Study Design

- 0, 2, 10, and 50 ppb in Purina 5K96 (soy-, alfalfa-free) diet to NCTR CD (Sprague-Dawley) rats

- Dosing (reproductive phase, terminated PND 140 in all generations)
  - F_0: from 28 days prior to mating to PND 140
  - F_1, F_2: from conception to sacrifice at PND 140
  - F_3: from conception through weaning at PND 21
  - F_4: no exposure

- 35 or 40 (40 in F_2 only) Breeding pairs per dose group, animals from 25 litters per dose group randomly selected for evaluation

Multigeneration Dosing Scheme



* F_4 generation was mated as F_0 to F_3 to produce F_5 litters

Approximate Ingested Dose
(Mean μg EE_2/kg body weight ± SEM)

	Dietary Concentration Ethinyl Estradiol		
	2 ppb	10 ppb	50 ppb
Males	0.1 ± 0.01	0.7 ± 0.04	3.9 ± 0.2
Females	0.2 ± 0.01	1.1 ± 0.1	5.8 ± 0.3

Comparison with human EE_2 exposure from oral contraceptives

- Approximate daily dose of EE_2 from oral contraceptives: 0.3 – 0.6 μg/kg
- Bioavailability is lower in rats than in humans due to more extensive metabolism
- Under the conditions of the present study, **serum levels were not measurable** in rats using an LC/MS method with a limit of detection of 10 pg/ml (Twaddle *et al*., J. Chromatogr. B 793: 309 – 315, 2003)
  - A 1 mg/kg gavage dose gave a C_{max} of 900 pg/ml and an AUC of 2800 pg x h / ml with a $t_{1/2}$ of 6 h in female rats
- For comparison, a pharmacokinetic study in premenopausal women with a single dose of 1.1 μg EE_2/kg gave a C_{max} of 245 pg/ml and an AUC of 2,365 pg x h / ml with a $t_{1/2}$ of 17 h (Scheffler et al., Clin. Pharmacol. Ther. 65: 483 – 490, 1999).

Body Weight and Food Consumption

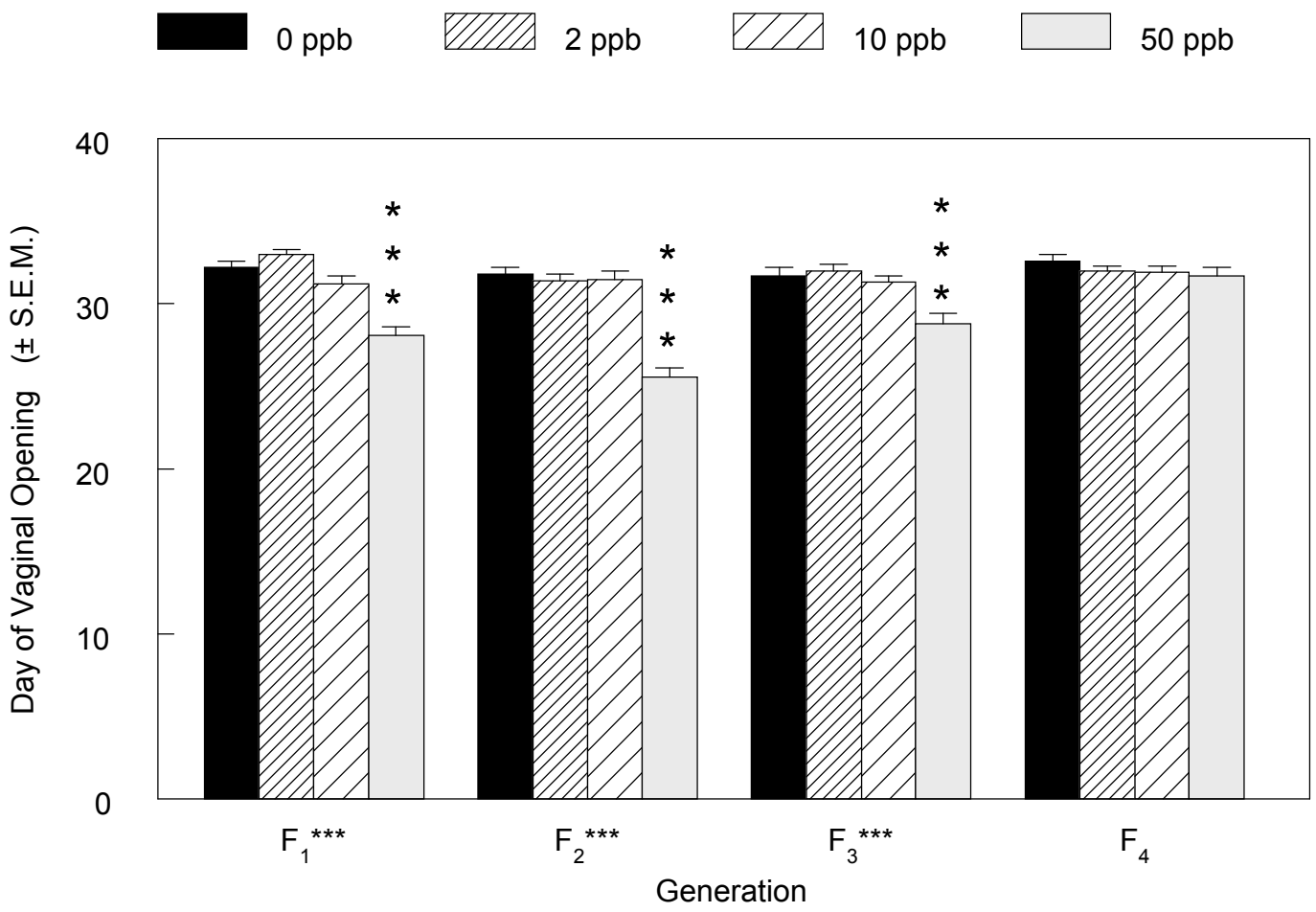
Endpoint	Generation				
	F_0	F_1	F_2	F_3	F_4
Body Weight					
Females					
Preweaning	NA	↓ (50)	↓ (50)	↓ (50)	-
Postweaning	↓ (10, 50)	↓ (50)	↓ (50)	-	-
Males					
Preweaning	NA	↓ (50)	↓ (50)	↓ (50)	-
Postweaning	↓ (50)	↓ (50)	↓ (2, 10, 50)	-	-
Feed Consumption					
Females	↓ (2)	-	↓ (50)	↑(10, 50)	-
Males	↓ (2, 10)	↓ (2)	↓ (2, 10, 50)	-	-

Ethinyl Estradiol Multigeneration Study

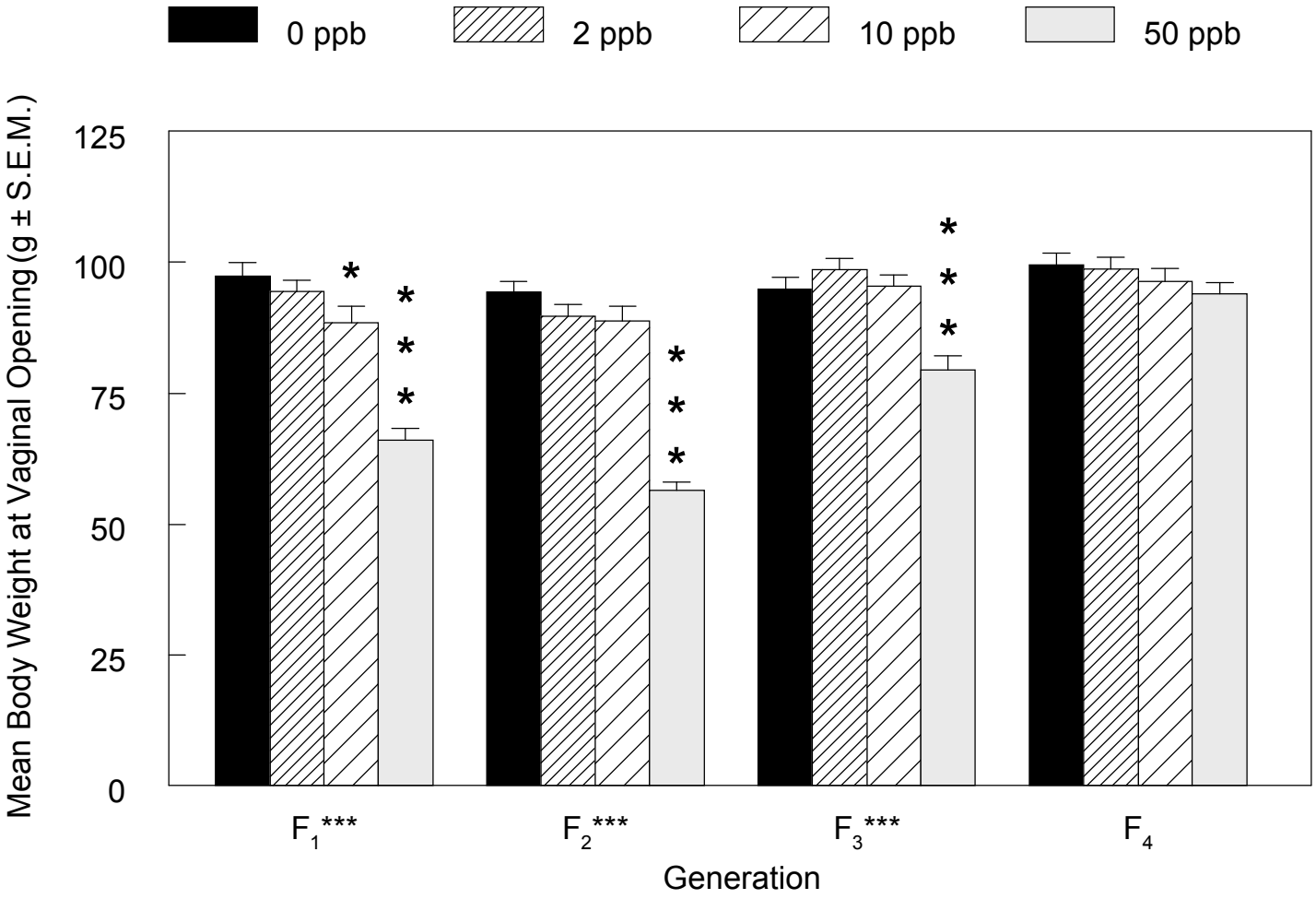
Endpoint	F_0	F_1	F_2	F_3	F_4
Accelerated Vaginal Opening	NA	50	50	50	-
Aberrant/prolonged cycles 5 wks old	NA	2, 10, 50	50	-	-
Aberrant cycles 20 wks old	-	-	-	-	-
Renal tubule mineralization, Males PND 140	-	50	50	-	-
Male mammary hyperplasia PND 140	50	2, 10, 50	10, 50	50	-

NA = not applicable; "-" = no significant effect

Age at Vaginal Opening



Body Weight at Vaginal Opening

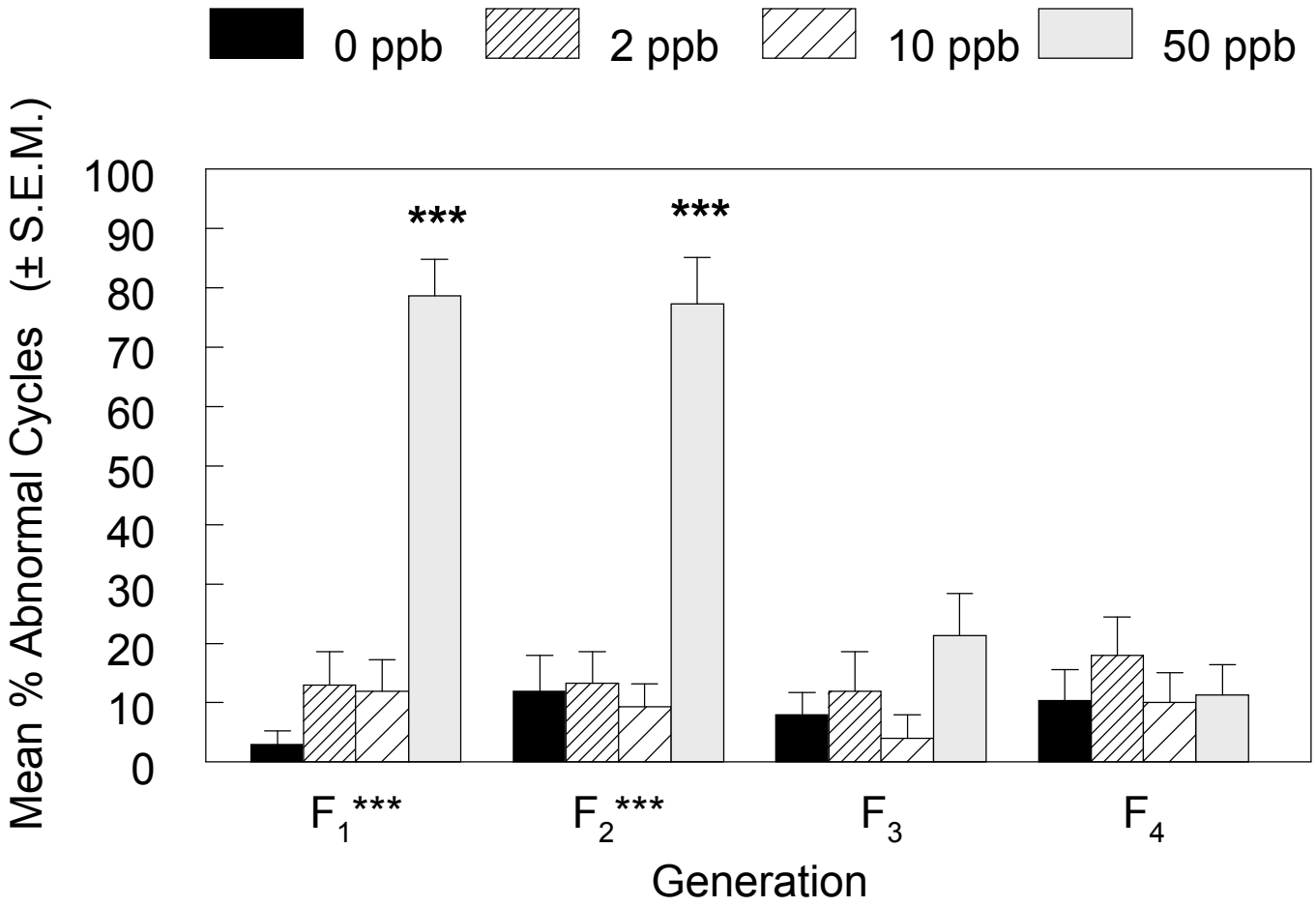


Vaginal Cytology Data from EE_2 Multigeneration Study

- F_1-F_4: 14 consecutive days from 3 days after vaginal opening
- F_0-F_4: 10 consecutive days prior to necropsy (PND 130-140, breeders, after delivering and nursing litters)
- In subsequent slides, abnormal cycle defined as 4 or more days of diestrus or 3 or more days of estrus in a 5 day period

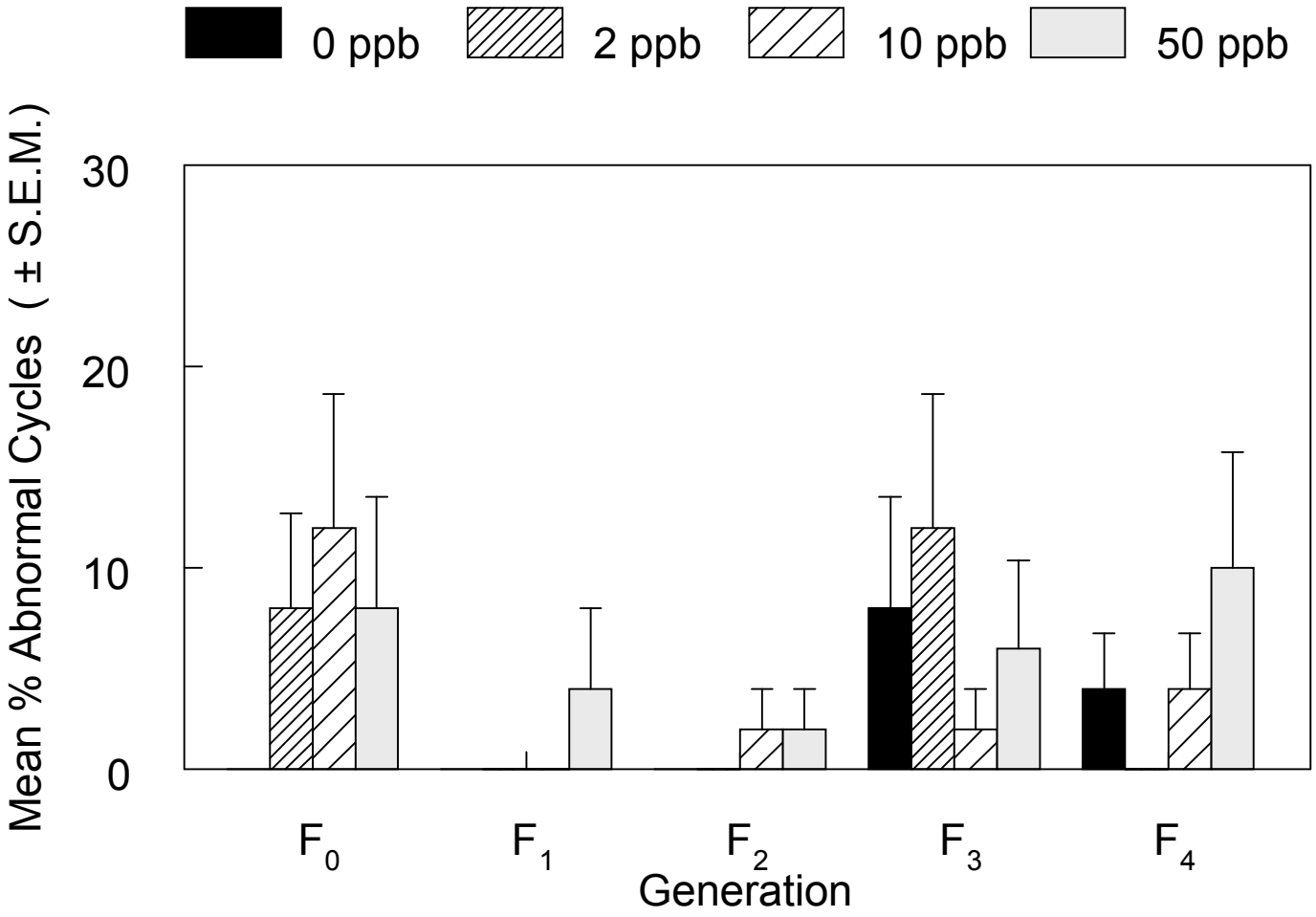
% Abnormal Cycles - Estrus and Diestrus

After Vaginal Opening



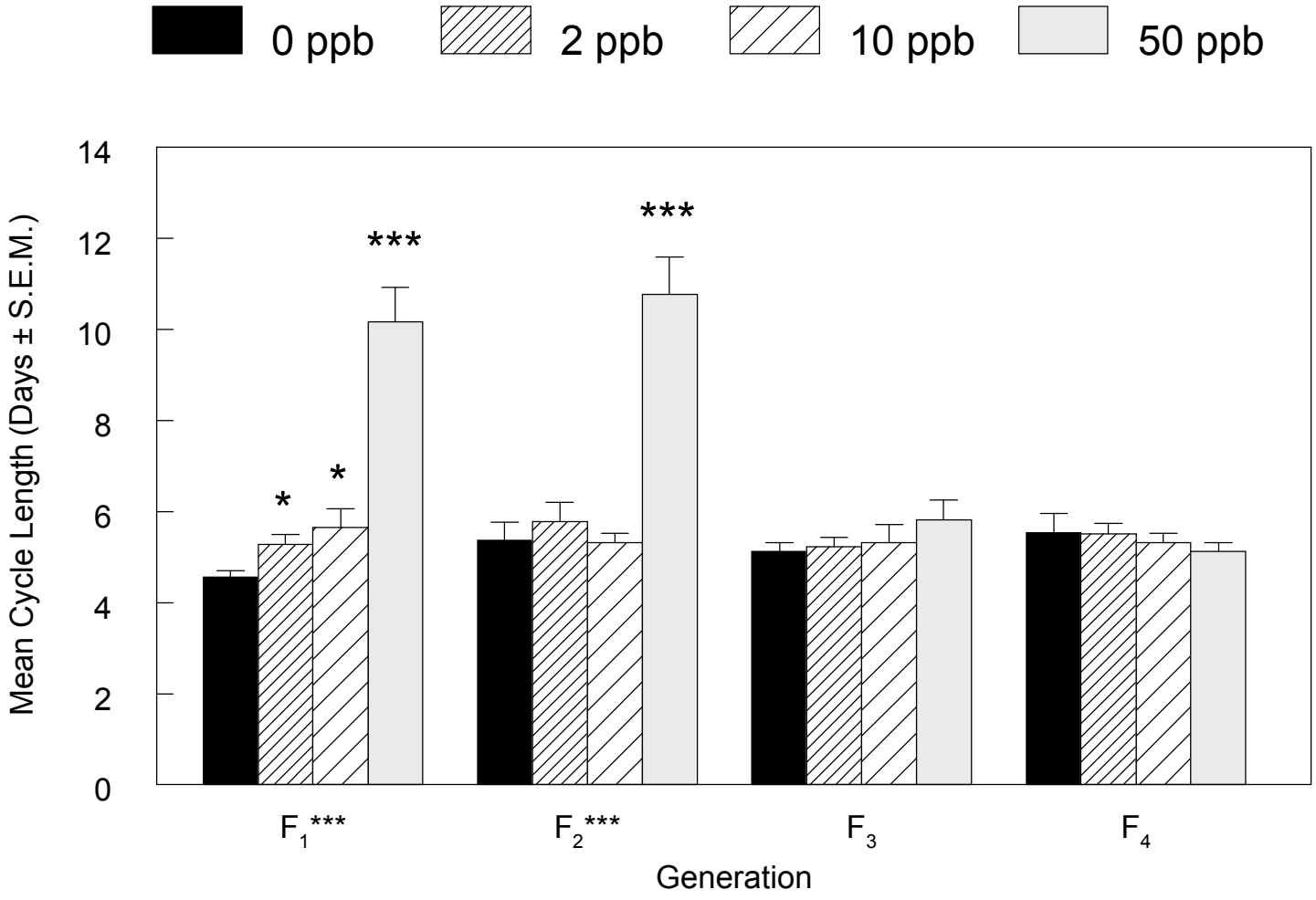
% Abnormal Cycles - Diestrus and Estrus

Before Sacrifice



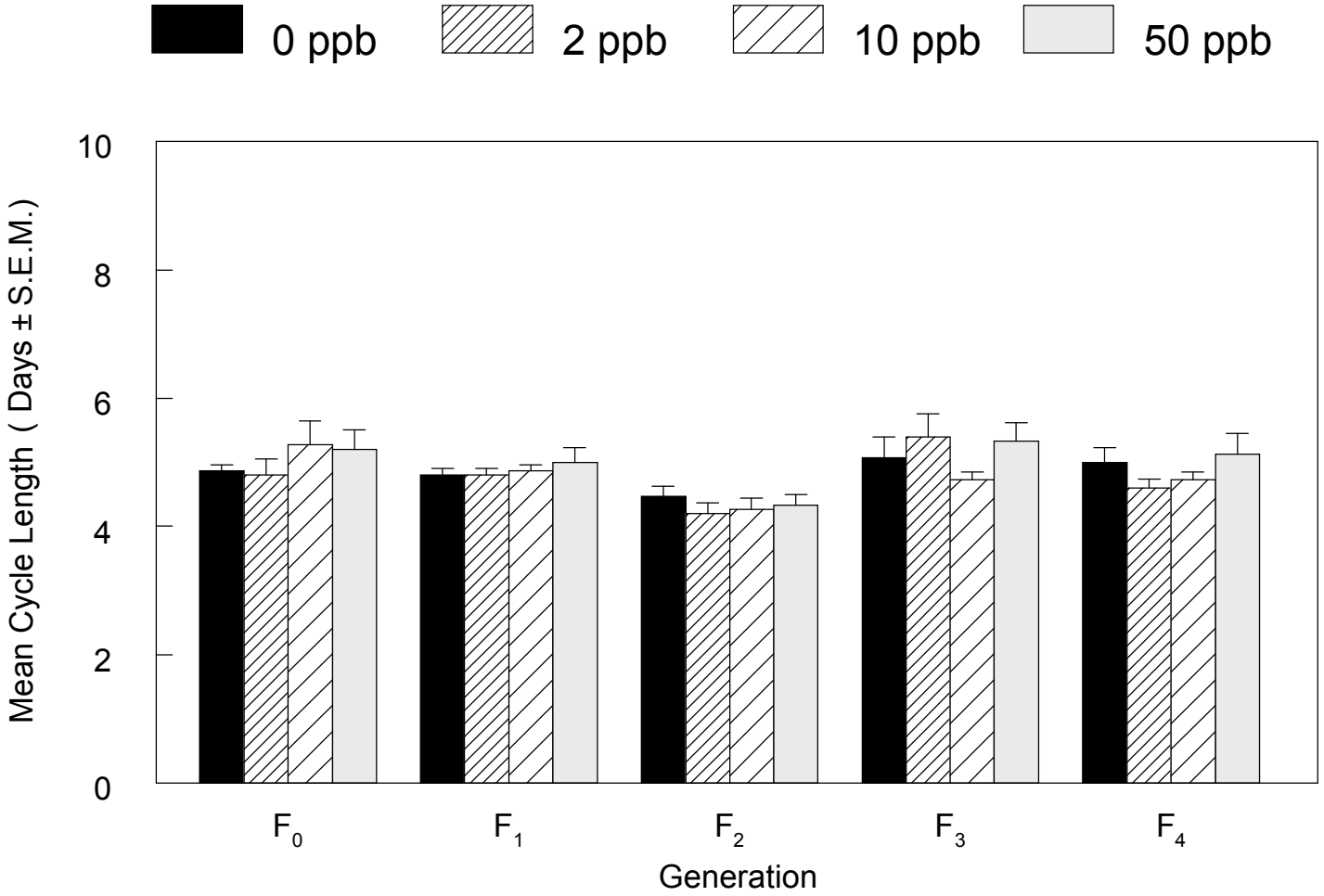
Length of Cycle

After Vaginal Opening



Length of Cycle

Before Sacrifice

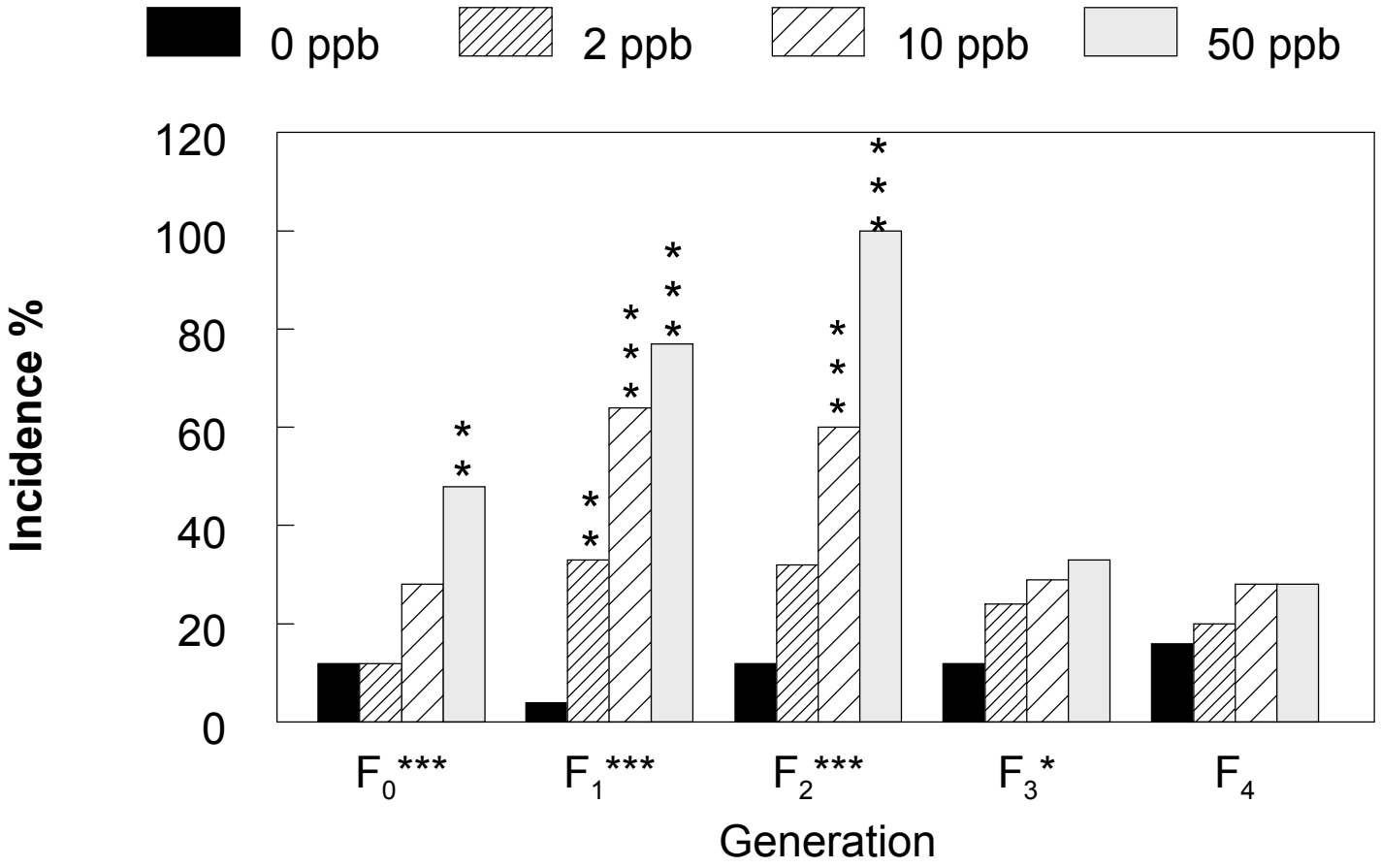


Male endpoints

- No consistent effects on spermatogenesis
- No consistent effects on markers of male puberty
- No effects on prostate weights or histology; no consistent effects on weights or histology of other male reproductive tract organs
- Stimulation of male mammary gland hyperplasia and mineralization of renal tubules

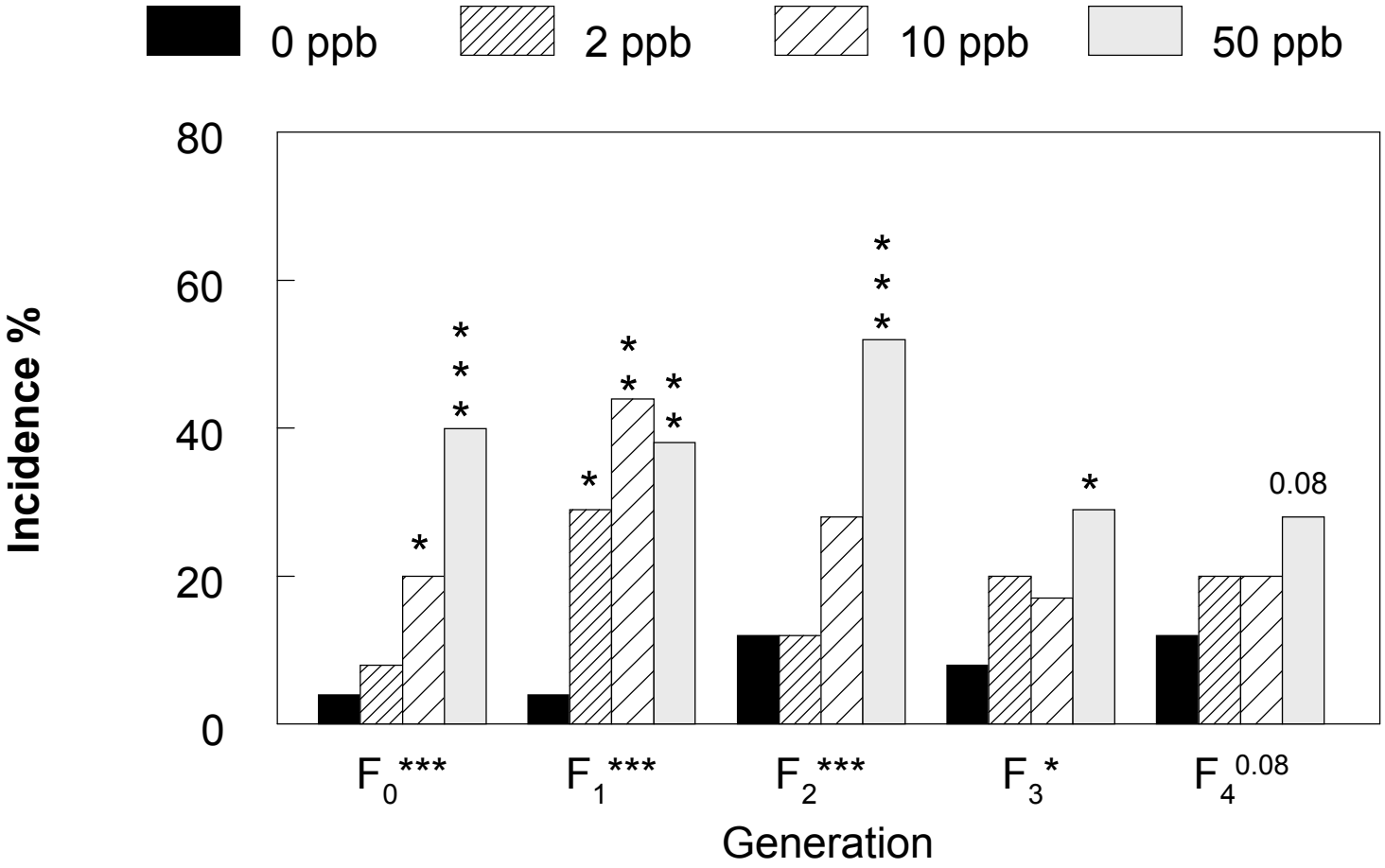
Male Mammary Gland Hyperplasia

Alveolar/Ductal, PND 140



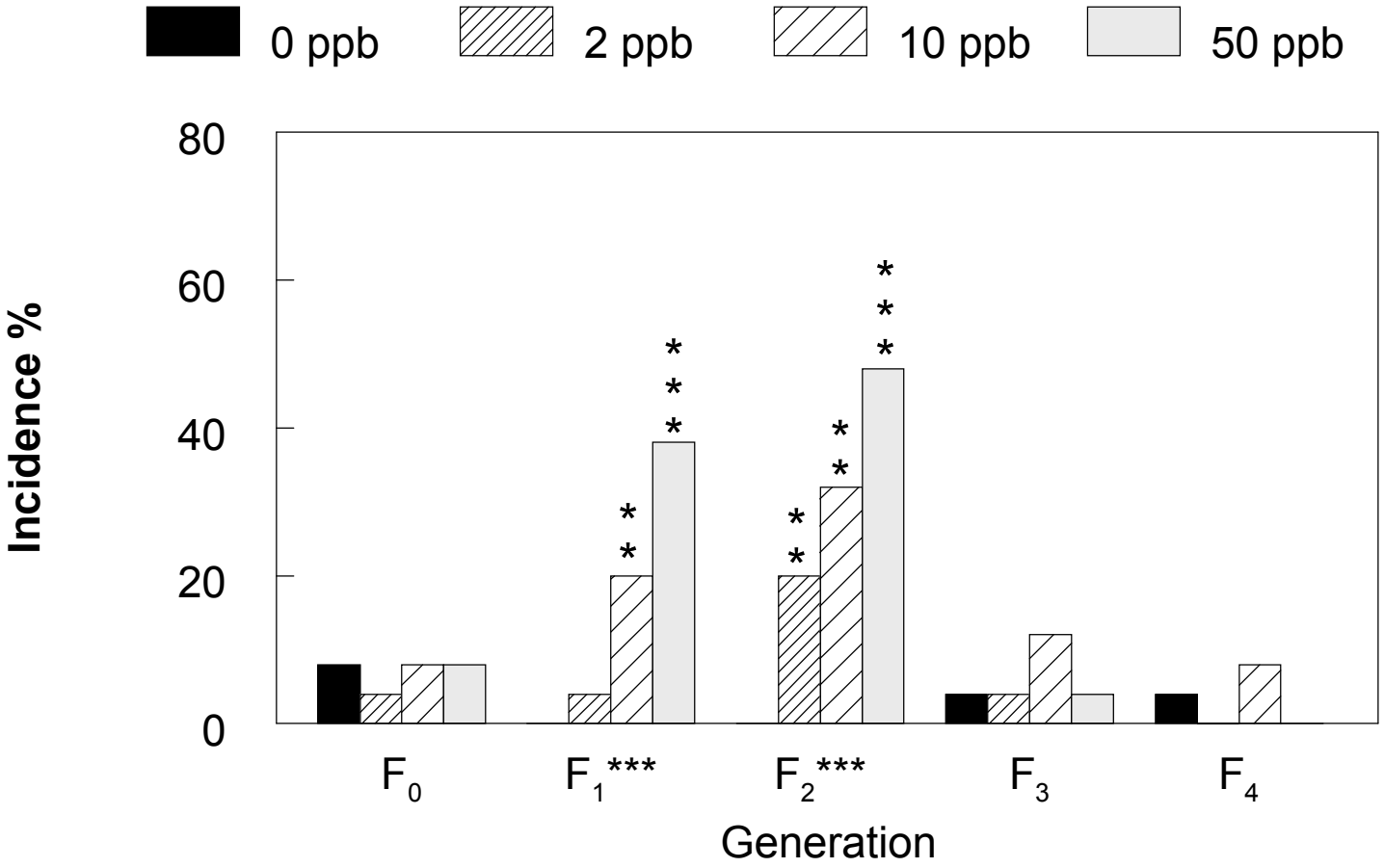
Male Mammary Gland Hyperplasia

Alveolar, PND 140



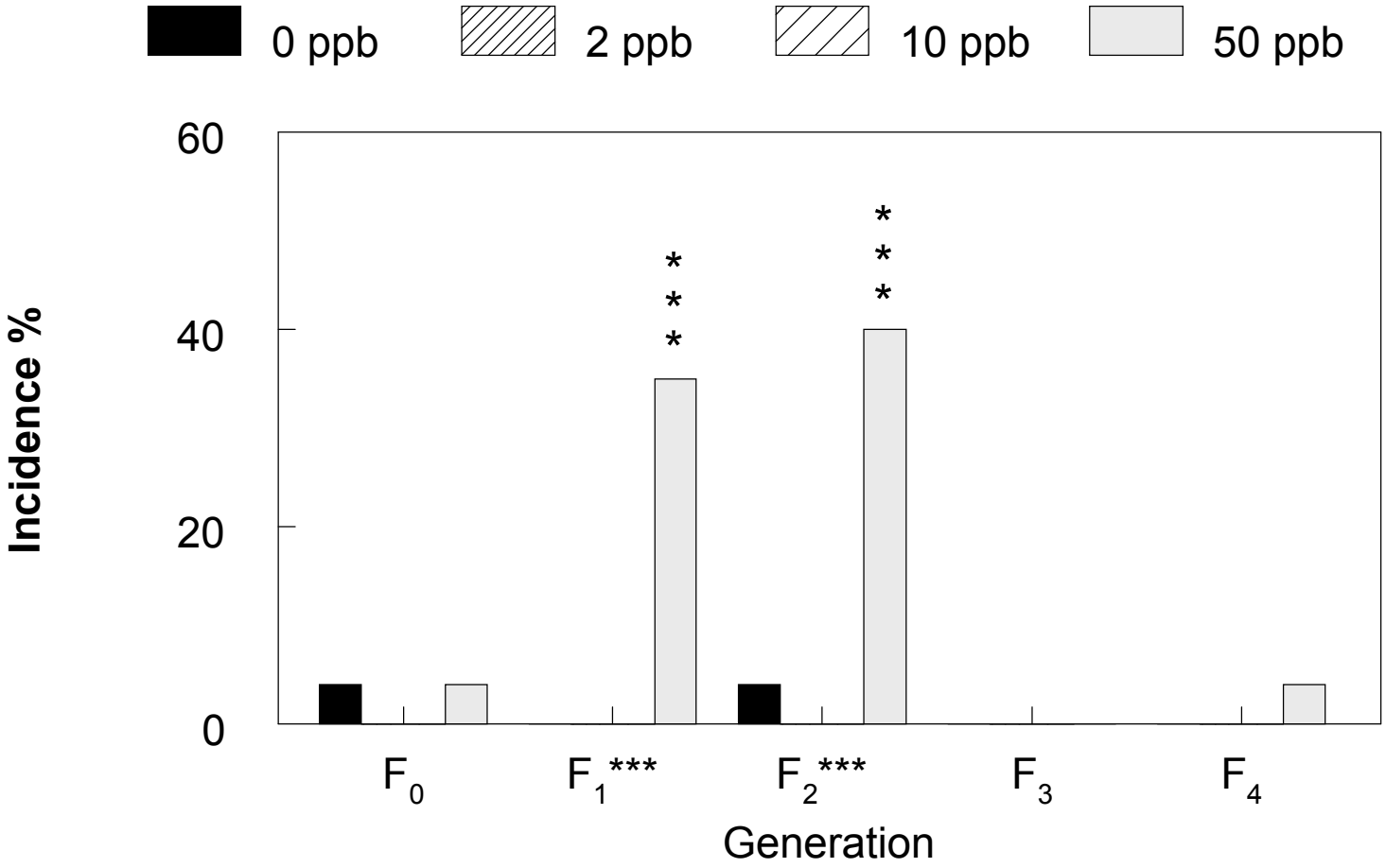
Male Mammary Gland Hyperplasia

Ductal, PND 140



Male Kidney

Renal Tubule Mineralization, PND 140



Conclusions (1): Multigeneration Study (TR 547)

- Reproductive toxicity evidenced by:
  - Accelerated time and/or lower body weights at vaginal opening (F_1, F_2, F_3)
  - Increased aberrant cycles and/or length of cycle (F_1, F_2)
  - Male mammary gland alveolar/ductal hyperplasia (F_0-F_3)

Conclusions (2): Multigeneration Study (TR 547)

- Other effects
  - Increased renal tubule mineralization in males (F_1 and F_2)
  - Decreased body weight gains in both sexes (F_0, F_1, F_2, F_3)
- Effects more prominent in continuously exposed generations
- Majority of effects at the high dose tested (50 ppb), but some significant effects (F_1 : male mammary hyperplasia, prolonged cycle; F_2 : male body weight) were observed at the lowest dose (2 ppb)
- With the exception of marginal increase in male mammary alveolar hyperplasia, no evidence for carryover or magnification of effects